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Who Actually Receives the Dose? A Cell-State-Aware Targeting Model for Spatially Heterogeneous Tumor Microenvironments and Treatment-Resistant Cellular Niches

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ABSTRACT

Nanomedicine performance is commonly summarized through administered dose, blood pharmacokinetics, organ biodistribution, or average tumor accumulation. These measurements are essential but cannot establish which vascular, stromal, immune, or malignant-cell states encounter a carrier, internalize it, release its payload, or achieve pharmacologically relevant intracellular exposure. This article develops the proposed Cell-State-Aware Targeting Model as an original non-empirical architecture for representing nanocarrier delivery across spatially heterogeneous tumor microenvironments. The model treats delivery as a sequence of conditional gates encompassing systemic availability, vascular proximity and entry, interstitial transport, cellular contact, receptor accessibility, internalization, intracellular trafficking, payload release, and state-specific biological action. Its primary analytical unit is a cell state at a defined spatial location and time rather than an averaged tumor, lesion, or cell type. Particular attention is given to resistant niches and treatment-induced state transitions that may alter target expression, accessibility, trafficking, or payload sensitivity after therapy begins. Proposed outputs include cell-state active-payload dose, target-state coverage, exposure dispersion, resistant-niche minimum coverage, off-target compartment capture, effective intracellular-delivery fraction, spatial exposure–target concordance, and transition-robust coverage. Validation would require coordinated spatial omics, multiplex imaging, quantitative pathology, carrier-specific localization, intracellular trafficking measurements, payload-state analysis, and functional assays. The framework is intended to organize mechanistic questions, experimental design, and evidence reporting rather than provide validated predictions or clinical treatment rules. Its central boundary is that tumor accumulation, cellular uptake, intracellular release, and therapeutic consequence are related but non-equivalent endpoints whose relationships remain conditional on formulation, disease context, biological state, measurement method, and intended decision use.

Key words: *Nanomedicine, Targeted drug delivery, Biological barriers, Intracellular release, Biodistribution, Protein corona*

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INTRODUCTION

Targeted cancer delivery aims to increase therapeutically useful exposure in diseased tissue while limiting exposure elsewhere. However, “tumor delivery” often conflates distinct events, including systemic circulation, vascular entry, tissue retention, cellular contact, internalization, intracellular trafficking, payload release, and pharmacological action. Increased tumor-associated signal therefore does not necessarily demonstrate delivery to

the intended cellular recipients. Biological barriers, tumor heterogeneity, and translational gaps remain major constraints on targeted-delivery claims [1].

The challenge extends beyond particle design. Development must connect formulation properties, manufacturing reproducibility, biological performance, safety, patient selection, and clinical positioning. A formulation that accumulates in a preclinical tumor may still fail to expose disease-sustaining cell states, while a targeting mechanism observed under one vascular, stromal, or immune configuration may not transfer to another. Average tumor uptake should therefore be treated as one component of a multistep delivery chain rather than as a surrogate for cellular dose or translational readiness [2].

Nanoparticle engineering enables control over size, shape, surface chemistry, elasticity, payload, ligand presentation, and release behaviour. Yet the biological consequences of these variables depend on protein adsorption, systemic clearance, vascular phenotype, tissue architecture, target accessibility, uptake pathways, and intracellular processing. A compositionally uniform carrier may consequently produce highly unequal exposure across vascular regions, stromal structures, immune populations, malignant-cell states, and intracellular compartments [3].

This Original Cell-State Targeting Model Article proposes the Cell-State-Aware Targeting Model (CSATM) to address a more demanding question: *who actually receives the dose?* CSATM uses the cell state at a defined location and time as its primary analytical unit and separates established mechanisms and measurements from proposed relationships, metrics, and decision rules. It is intended as a conceptual architecture—not an exhaustive review, validated predictive model, clinical recommendation, or regulatory standard—for evaluating whether nanocarriers reach intended cell states, interact with alternative cellular compartments, remain effective across treatment-induced transitions, and deliver active payload to the intracellular site required for action.

Why average tumor uptake obscures cellular dose distribution

Average tumor uptake relates tumor-associated carrier or payload signal to tissue mass, injected dose, imaging intensity, or another aggregate denominator. These measures can show that material reached or remained associated with a tumor, but they cannot establish its route of entry, extracellular or intracellular location, recipient-cell identity, payload state, or distribution among cells. Vascular entry itself cannot be attributed automatically to passive movement through endothelial gaps because active transendothelial processes may also contribute [4].

Spatially restricted vascular entry further limits tumor-wide averages. Entry-permissive endothelial regions may generate local exposure territories around selected vessels while leaving other regions poorly supplied. Specialized endothelial populations have been associated with nanoparticle entry, indicating that vascular density alone does not define delivery opportunity [5]. Tumors with similar average accumulation may therefore differ substantially in the number, location, and phenotype of permissive vascular sites.

Aggregate measurements also conceal redistribution among cellular compartments. Nanocarriers may be retained by perivascular cells, extracellular structures, fibroblast-associated regions, macrophages, or other immune populations before reaching malignant cells. Tumor barriers can determine which macrophage populations encounter nanoparticles, demonstrating unequal exposure even within one broad cellular category [6]. High tumor signal may therefore reflect concentrated uptake by phagocytic cells rather than broad malignant-cell delivery. Depending on the payload and intended mechanism, such uptake may be harmful, neutral, or therapeutically useful.

CSATM consequently distinguishes tumor accumulation, cellular contact, carrier internalization, released payload, and pharmacologically active intracellular dose. Divergence among these quantities may result from vascular heterogeneity, transport limitations, extracellular binding, matrix structure, protein-corona effects, cellular sequestration, receptor accessibility, endocytic routing, inadequate endosomal escape, incomplete release, or state-dependent sensitivity. The model does not assign one universal mechanism but requires identification of the point at which an aggregate measurement no longer supports a claim about the intended cellular recipient.

Tumor spatial organization and cellular-state heterogeneity

A cell state is defined as a context-dependent molecular, phenotypic, and functional configuration within a nominal cell type. A spatial niche comprises the neighbouring cells, extracellular structures, vascular features, biochemical conditions, and treatment history surrounding that state. Although dissociation-based single-cell analysis identifies cellular diversity, it removes much of the original tissue organization. Integration with spatial transcriptomics has revealed localized malignant, stromal, and immune programmes that cannot be reconstructed

reliably from bulk abundance alone [7]. CSATM therefore treats cell state and location as jointly necessary descriptors.

Tumors are structured ecosystems rather than uniform mixtures of malignant and non-malignant cells. Multiplexed imaging has identified compartmentalized and mixed tumor–immune organizations with distinct cellular boundaries and interactions [8]. The same malignant or immune-cell state may therefore experience different exposure depending on whether it is vessel-adjacent, stromally enclosed, positioned at a tumor–immune interface, or located in a poorly perfused region. These arrangements may affect transport, sequestration, target accessibility, release, and response, although such effects require direct measurement.

The cellular-neighbourhood concept extends analysis beyond isolated cells. Coordinated malignant, immune, stromal, and vascular neighbourhoods have been identified at invasive tumor boundaries [9]. A nanocarrier may therefore encounter a sequence of interacting states, such as endothelial entry, perivascular macrophage uptake, fibroblast-associated restriction, extracellular binding, and eventual malignant-cell contact. CSATM represents these neighbourhoods as connected spatial nodes that may alter the probability of completing subsequent delivery steps.

Spatial and cellular-state heterogeneity are related but distinct. One cell state may occupy several niches, while one niche may contain several states, and both may change during tumor growth or treatment. CSATM therefore interprets delivery through four coordinates: cell identity, cell state, spatial niche, and time. This prevents bulk tumor averages, cell-type averages, or static baseline maps from being treated as complete descriptions of cellular dose.

Proposed cell-state-aware targeting model

Delivery-system design should begin with the biological requirements of therapeutic action and work backward through the barriers that must be crossed. A biology-informed framework asks which cells require exposure, where they are located, which extracellular and intracellular barriers separate them from the administered formulation, and what payload state is required for activity [10].

CSATM extends this logic by making the spatially situated cell state the central recipient and representing delivery as a conditional sequence rather than a single targeting event. **Figure 1** presents the proposed spatial map of nanocarrier exposure across tumor cell states and resistant niches, integrating the model's principal components, evidence relationships, uncertainties, and decision boundaries.

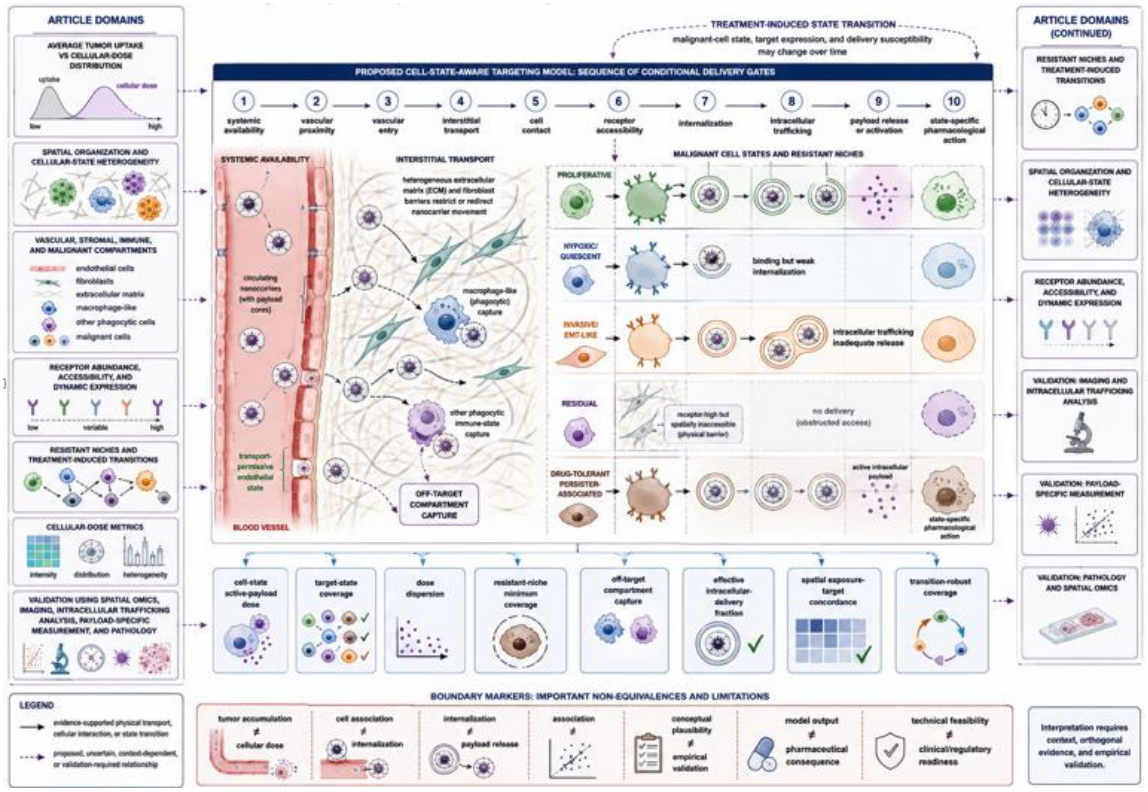

Figure 1. Spatial Map of Nanocarrier Exposure across Tumor Cell States and Resistant Niches.

The gates are sequential but interdependent: systemic disposition limits tumor access; vascular and stromal conditions shape entry and transport; cellular contact and receptor accessibility affect engagement; and intracellular trafficking and release determine pharmacological availability. Because nanomedicine performance depends on interacting biological and product-related barriers, no single gate is sufficient [11].

CSATM also separates carrier state from payload state. Nanocarriers may acquire a protein corona, aggregate, degrade, exchange components, or release payload before or after cellular uptake. Likewise, measured signal may represent surface association, endosomal retention, cytosolic access, organelle localization, released drug, or downstream action. These stages require distinct measurements and controls [12]; total fluorescence or carrier-associated signal cannot therefore be assumed to represent active intracellular dose.

Conceptually, CSATM forms a spatial-temporal graph in which each node records cell identity, state, niche, target status, delivery status, and time. Edges represent adjacency, transport, interaction, or state transition. The framework connects systemic disposition, tissue entry, and intracellular processing without treating them as equivalent endpoints [10-12].

Proposed outputs include active-payload dose by cell state, target-state coverage, dose dispersion, resistant-niche minimum coverage, off-target capture, effective intracellular-delivery fraction, spatial exposure–target concordance, and coverage after treatment-induced state transitions. These are analytical categories, not validated thresholds or clinical rules.

Vascular, stromal, immune, and malignant-cell compartments

The vascular compartment cannot be characterized by vessel density or perfusion alone. Tumor endothelial cells occupy heterogeneous molecular and functional states, and individual vessels may differ in their capacity to support nanocarrier entry [13]. CSATM therefore records endothelial state, vessel structure, flow, perivascular organization, and observed crossing events.

The stromal compartment includes extracellular matrix, fibroblast states, pericytes, interstitial pressure, and fluid pathways. Cancer-associated fibroblasts are heterogeneous and may differ in matrix production, inflammatory signalling, antigen presentation, and cellular interactions [14]. Rather than assigning one "stromal barrier" value, CSATM evaluates whether particular stromal states restrict, retain, redirect, degrade, or release a carrier.

Immune cells may act as sinks, transport intermediates, therapeutic targets, toxicity mediators, or sources of pharmacological amplification. Human tumors contain diverse immune states shaped by lineage and local context [15]. Analysis should therefore identify which immune states receive the carrier, whether uptake is intracellular, whether the payload remains active, and whether sequestration supports or competes with the intended mechanism.

Malignant cells are likewise represented as heterogeneous states, including proliferative, invasive, hypoxic, quiescent, residual, and drug-tolerant populations. These states may differ in vascular proximity, receptor accessibility, uptake, intracellular processing, and drug sensitivity. Because vascular, stromal, immune, and malignant compartments influence one another, CSATM treats them as interacting spatial systems rather than fixed or independent barriers [6].

Table 1 organizes the evidence, constructs, relationships, uncertainties, and boundary conditions required for definitions, components, relationships, and intended uses in who actually receives the dose?.

Table 1. Definitions, components, relationships, and intended uses in Who Actually Receives the Dose?

Component or scientific dimension	Problem addressed	Inputs or determinants	Proposed mechanism or relationship	Expected contribution	Evidence required	Failure or uncertainty risk	Boundary statement
Whole-tumor accumulation	Aggregate signal does not identify cellular recipients or intracellular payload state.	Administered dose, circulation, tumor-associated carrier or payload signal, tissue mass, sampling time.	Evidence-supported distinction: tumor association may arise through several entry, retention, and cellular-	Provides an upstream exposure descriptor without being treated as cellular dose.	Quantitative biodistribution combined with spatial and cell-resolved localization.	Label detachment, extracellular retention, necrotic trapping, vascular contamination, and averaging across	Whole-tumor accumulation does not establish malignant-cell delivery, intracellular release, or pharmacological action.

			capture pathways [4].		heterogeneous regions.		
Vascular-entry state	Vessel abundance does not identify where or how carrier entry occurs.	Endothelial phenotype, vessel structure, flow, perivascular cells, carrier properties, local permeability.	Proposed CSATM relationship: entry probability is conditioned by spatially localized endothelial states; specialized entry-associated endothelial cells provide supporting evidence [5].	Identifies heterogeneous entry opportunities and poorly supplied territories.	Intravital or three-dimensional localization linking endothelial state to carrier-crossing events.	Colocalization may be mistaken for causation; entry mechanisms may be carrier- and model-specific.	An endothelial marker cannot be used predictively until transport relevance is validated for the formulation and context.
Stromal transport state	A single stromal-density measure cannot explain transport through heterogeneous tissue.	Fibroblast state, matrix composition, matrix architecture, interstitial pressure, tissue fluid flow, degradation.	Proposed CSATM relationship: stromal states can restrict, redirect, retain, or transform carrier movement; fibroblast heterogeneity is evidence-supported [14].	Separates physical transport effects from generic "stroma-rich" classifications.	Spatially matched matrix, fibroblast-state, and carrier-distribution measurements.	Matrix markers may not represent functional transport; tissue preparation may distort geometry.	Stromal-state identity alone does not establish whether delivery is promoted or restricted.
Immune-cell capture	Total immune uptake obscures the identity and intended role of recipient immune states.	Immune-cell state, phagocytic activity, protein corona, particle size, surface chemistry, local exposure.	Evidence-supported and proposed relationship: tumor barriers redistribute macrophage access [6], while CSATM classifies capture by intended function.	Distinguishes therapeutic immune targeting from unintended sequestration.	State-resolved carrier localization, intracellular payload analysis, and functional immune assays.	Immune uptake may be labelled incorrectly as uniformly beneficial or harmful.	Immune-cell capture must be interpreted relative to payload, target state, and intended mechanism.
Malignant-cell state	"Tumor-cell uptake" averages across biologically different malignant populations.	Lineage, differentiation, proliferation, hypoxia, stress, receptor state, treatment history, spatial niche.	Proposed CSATM relationship: malignant states differ in access, internalization, trafficking, release, and payload sensitivity.	Directs analysis toward intended and treatment-resistant recipients.	Spatial state annotation combined with carrier, payload, and response measurements.	State definitions may be unstable, technology-dependent, or tumor-specific.	A transcriptomic or phenotypic state is not automatically a delivery phenotype.
Cell-contact dose	Tumor accumulation does not establish that a	Spatial proximity, extracellular transport,	Evidence-supported distinction: ligand-coated	Establishes the earliest cell-specific	Microscopy or analytical methods distinguishing	Surface association may be	Cell contact is not evidence of uptake, release, or active

	carrier contacts the intended cell surface.	ligand availability, receptor accessibility, local competition.	particle accumulation may correspond to limited direct cancer-cell delivery [16].	exposure endpoint.	surface contact from extracellular background.	confused with internalization.	intracellular exposure.
Receptor accessibility	Receptor abundance can be high while target sites remain physically inaccessible.	Receptor density, tissue depth, binding affinity, internalization rate, carrier size, competing ligands.	Evidence-supported distinction: strong peripheral binding can limit deeper penetration [17].	Prevents receptor-expression-only targeting claims.	Spatially resolved accessible-target assays, binding measurements, and penetration analysis.	Fixed tissue staining may overestimate in vivo access; affinity may worsen distribution.	Expression does not establish access, engagement, or intracellular delivery.
Intracellular trafficking and release	Internalized carrier does not establish that active payload reaches its required site.	Endocytic route, vesicular processing, endosomal escape, degradation, stimulus sensitivity, release kinetics.	Evidence-supported distinction and proposed CSATM gate: internalization, trafficking, and intracellular localization require separate measurement [12].	Connects carrier uptake to payload availability and subcellular action.	Orthogonal carrier- and payload-specific assays with organelle and chemical-state resolution.	Fluorescent or elemental labels may persist after carrier transformation or payload loss.	Carrier localization cannot substitute for evidence of released and pharmacologically active payload.
Protein-corona-conditioned identity	The biological identity encountered by cells may differ from the manufactured surface identity.	Protein composition, concentration, exposure time, disease state, surface chemistry, shear, degradation.	Proposed CSATM modifier: corona formation may change clearance, receptor engagement, cell capture, and intracellular routing.	Connects systemic nano–bio interaction to state-specific delivery.	Context-matched corona characterization and functional uptake or transport studies.	Ex vivo corona preparations may not reproduce in vivo composition or kinetics.	Nominal ligand presentation does not prove preservation of targeting function in vivo.
Resistant-niche coverage	Mean exposure can obscure poorly supplied states that sustain residual disease.	Resistant-state identity, niche location, target state, carrier access, payload sensitivity, treatment timing.	Original proposed construct: prioritize the least adequately exposed prespecified resistant niche rather than only the tumor-wide mean.	Makes low-coverage, high-consequence regions visible.	Longitudinal spatial-state and active-payload measurement linked to functional persistence.	Resistant states may be misclassified or may change during treatment.	No universal resistant-niche threshold is proposed.
Transition-robust coverage	A baseline delivery map may become obsolete after	Treatment, time, lineage, stress response,	Original proposed construct: evaluate	Introduces time into targeting evaluation.	Repeated or longitudinal sampling with matched state,	Sampling intervals may miss transient states;	Transition robustness is investigational and does not

	therapy alters cellular states.	target expression, altered trafficking, lesion evolution.	whether coverage persists after clinically relevant state transitions.		target, and delivery measurements.	repeated tissue access may be limited.	imply prevention of resistance.
Intended use of CSATM	Targeting claims lack a common structure linking aggregate exposure to cellular pharmacology.	Intended payload, target population, tumor context, measurement platform, decision purpose.	Original proposed synthesis: integrate compartments, gates, states, spatial relationships, and time into one evidence architecture	Supports hypothesis generation, experimental design, reporting, and falsifiable evaluation.	Prospective testing across formulations, models, lesions, and decision contexts.	Model complexity, measurement burden, incomplete state definitions, and domain shift.	CSATM is not a validated predictor, clinical recommendation, regulatory standard, or deployment-ready system.

Receptor abundance, accessibility, and dynamic expression

Receptor abundance alone does not establish targeting success. Targets may be inaccessible because of poor perfusion, stromal barriers, distance from permissive vessels, or high-affinity binding near tissue surfaces that limits deeper penetration [17]. Ligands may increase local association without overcoming vascular, interstitial, or cellular barriers, and only a limited fraction of tumor-associated nanoparticles may reach malignant cells directly [16]. CSATM therefore evaluates coverage of intended cell states rather than total tumor uptake.

Accessibility may also differ among lesions and over time. Molecular imaging has demonstrated lesion-level target heterogeneity [18], while therapy may alter receptor expression, localization, internalization, or accessibility. CSATM consequently distinguishes total, accessible, engaged, internalization-competent, and temporally stable target. Receptor expression is not equivalent to cellular access or active intracellular dose.

Resistant niches and treatment-induced state transitions

Resistant niches contain spatially situated states that persist or adapt during treatment, including hypoxic, quiescent, invasive, stem-like, stress-adapted, and immune-protected populations. Residual disease can contain several biologically distinct states rather than one uniform resistant population [19].

Therapy can reshape malignant and microenvironmental populations, making pretreatment targeting maps unreliable [20]. Persister cells may also arise through different lineage programs [21]. CSATM therefore introduces transition-robust coverage: the extent to which delivery remains adequate across clinically relevant state changes. This construct requires longitudinal validation and does not imply that carrier localization alone prevents resistance.

Cellular-dose metrics and coverage criteria

Cellular dose should be measured at the biological level required for action. Mean fluorescence or two-dimensional imaging may combine extracellular, surface-bound, vesicular, cytosolic, nuclear, and released-payload signals. Three-dimensional imaging can distinguish intracellular distributions [22], while organelle-resolved methods show that similar total uptake may produce different intracellular trafficking patterns [23].

CSATM defines the effective intracellular-delivery fraction as the proportion of cell-associated material reaching the location and chemical state required for activity. Coverage analysis may further identify well-supplied and poorly supplied cell states or lesions [24].

Proposed metrics include active-payload dose, target-state coverage, dose dispersion, worst-state coverage, resistant-niche coverage, off-target capture, intracellular-delivery fraction, exposure–target concordance, and transition-robust coverage. No universal thresholds are proposed. Each metric should specify its denominator, state definition, spatial unit, assay, time point, and uncertainty.

Table 2 organizes the evidence, constructs, relationships, uncertainties, and boundary conditions required for testable propositions, evidence requirements, and validation criteria for who actually receives the dose?.

Table 2. Testable propositions, evidence requirements, and validation criteria for Who Actually Receives the Dose?

Architecture layer or process stage	Core function	Information or material flow	Interaction with other components	Validation criterion	Potential failure mode	Human or experimental responsibility	Readiness boundary
P1: Aggregate-to-cellular distribution	Determine whether similar tumor accumulation conceals different cell-state exposure.	Whole-tumor signal is decomposed into compartment-, state-, and cell-level distributions.	Connects biodistribution to vascular entry, cellular contact, and intracellular payload.	Matched bulk and cell-resolved assays demonstrate whether accumulation predicts intended-state exposure; tumor-entry heterogeneity provides mechanistic precedent [4].	Bulk label reflects extracellular, vascular, stromal, or immune localization rather than intended cellular dose.	Investigators must prespecify the intended recipient state and validate signal attribution.	Similar bulk accumulation is not evidence of equivalent cellular delivery.
P2: Vascular-entry constraint	Test whether spatially restricted endothelial states govern downstream coverage.	Carrier crosses selected vascular regions and enters local tissue territories.	Conditions stromal transport, immune capture, and malignant-cell contact.	Endothelial state, observed crossing event, and downstream carrier distribution are measured in the same spatial specimen; entry-associated endothelial populations provide supporting evidence [5].	Endothelial colocalization is interpreted as causal transport; carrier remains intravascular.	Experimental teams must distinguish vessel association, crossing, and interstitial localization.	No vascular biomarker is ready for decision use without carrier-specific validation.
P3: Stromal and immune redistribution	Determine whether non-malignant capture increases tumor signal while reducing or redirecting malignant-cell delivery.	Carrier moves from vascular entry regions into matrix, fibroblast, and immune compartments.	Competes with or supports delivery to malignant states.	Quantify carrier and payload separately across stromal, immune, and malignant cells; macrophage redistribution evidence supports the need for state-resolved analysis [6].	All non-malignant uptake is classified as delivery failure despite intended immunomodulatory action.	Investigators must define whether each recipient compartment is intended, neutral, or competing.	Compartment capture has no universal beneficial or harmful interpretation.
P4: Accessibility beyond abundance	Test whether receptor expression predicts delivery only when spatial	Target information flows from expression mapping to accessible binding,	Interacts with vascular distance, stromal transport, affinity, carrier	Expression is compared with accessible-target measurement, carrier contact, internalization,	High expression is used as a surrogate for broad tissue penetration.	Assay designers must separate total receptor, accessible receptor, and functionally	Receptor abundance alone cannot support targeting readiness.

	access and penetration are preserved.	engagement, and uptake.	size, and internalization.	and intracellular payload; binding-site limitation provides evidence for the distinction [17].		engaged receptor.	
P5: Intracellular-delivery completion	Establish whether internalized carrier produces active payload at the required intracellular site.	Carrier proceeds through internalization, trafficking, release, and payload activation.	Links cellular exposure to pharmacological mechanism.	Orthogonal carrier, payload, organelle, and activity assays demonstrate completion of each required gate; organelle-resolved trafficking provides a methodological route [23].	Persistent label is mistaken for intact carrier, released payload, or activity.	Experimental teams must use label-stability controls and payload-specific chemical or functional assays.	Intracellular carrier localization is not evidence of active intracellular dose.
P6: Resistant-niche minimum coverage	Test whether mean dose hides inadequate exposure in prespecified high-consequence niches.	Cell-state dose distributions are summarized by both mean and minimum or lower-tail niche coverage.	Connects resistant-state maps to spatial delivery and intracellular payload.	Resistant-state identity, local exposure, payload state, and persistence are measured jointly; residual-state diversity supports the need for stratification [19].	The “worst” niche is selected retrospectively or defined from noisy markers.	Investigators must prespecify niche definitions and uncertainty procedures.	No universal minimum-coverage threshold is proposed.
P7: Transition-robust coverage	Test whether targeting remains aligned after treatment changes state composition or target expression.	Longitudinal state maps update the intended recipient set and delivery requirements.	Interacts with target dynamics, lesion evolution, and repeat dosing.	Before-and-after measurements demonstrate whether relevant state transitions alter access, internalization, or active payload; treatment-induced evolution supports longitudinal reassessment [20].	Sparse sampling misses transient states or attributes natural evolution to treatment.	Study teams must justify timing, sampling, comparability, and causal interpretation.	Transition robustness must be demonstrated for each treatment–carrier context.
P8: Cross-lesion transfer	Determine whether targeting observed in	Formulation and biological information are compared	Interacts with vasculature, stroma, immune capture, target	Matched analyses evaluate distribution	One lesion, model, or metastatic site is treated as	Translational teams must define the domain in which	Cross-lesion transfer is not assumed from

	one lesion class transfers to other lesions.	across primary, metastatic, and organ-specific environments.	accessibility, and payload release.	and cellular recipients across lesion types; primary-versus-metastatic differences demonstrate the relevance of this test [25].	representative of all disease.	any targeting claim is intended to hold.	formulation identity or primary-tumor data.
P9: Orthogonal validation	Determine whether cell identity, location, carrier, payload, and consequence are supported by independent methods.	Molecular, imaging, pathological, chemical, and functional evidence are integrated.	Validates all upstream and downstream CSATM layers.	Concordant evidence is obtained without collapsing disagreements among modalities.	Shared labels or preprocessing create artificial agreement; discordance is averaged away.	Analysts must maintain provenance, uncertainty, and modality-specific limitations.	Multimodal agreement supports measurement validity but not automatically clinical utility.
P10: Product and implementation stability	Test whether nominally similar material retains cell-state targeting across batches and biological contexts.	Characterized formulation attributes connect to systemic identity, delivery gates, and spatial outputs.	Links manufacturing, protein corona, stimuli-responsive release, and biological variability.	Prespecified critical attributes and matched biological assays show reproducibility of relevant cellular-dose distributions.	Batch similarity is judged only by bulk physicochemical or tumor-uptake measures.	Product-development teams must justify which attributes are critical to the intended cellular mechanism.	Analytical similarity does not establish biological, clinical, or regulatory equivalence.
P11: Human interpretation and escalation	Prevent model outputs from being treated as autonomous treatment decisions.	Evidence and uncertainty flow from experimental systems to expert interpretation.	Applies across metric definition, validation, domain transfer, and decision use.	Human reviewers can trace each output to measurements, assumptions, uncertainty, and applicable domain.	A composite score hides missing gates, weak evidence, or unmeasured resistant states.	Multidisciplinary reviewers must approve intended use and escalation boundaries.	CSATM remains a research and evidence-organizing architecture until prospective utility is established.

Validation using spatial omics, imaging, and pathology

Validation strategy

Validation should first establish whether proposed cell states and niches can be measured reproducibly in intact tissue. Multiplex pathology can preserve morphology while identifying cell populations, spatial relationships, and carrier-associated compartments [26], but cannot alone confirm functional state or distinguish extracellular attachment from intracellular delivery.

CSATM therefore requires orthogonal validation: molecular and phenotypic state definition, spatial localization, separate detection of carrier and payload, assessment of intracellular disposition, and measurement of mechanism-relevant effects [27]. Reference atlases may support annotation [28], but they cannot replace formulation-specific testing in the relevant lesion, model, dose, and sampling window.

Validation should progress from computational reproducibility and experimental testing of delivery gates to pharmaceutical, translational, and prospective clinical evaluation. Success at one level does not establish readiness at the next.

Limitations and translational implications

CSATM is limited by unstable cell-state definitions, incomplete tissue sampling, technical measurement biases, and persistent gaps between experimental models and human tumor biology [29]. It also cannot fully capture systemic factors such as clearance, protein corona formation, immune recognition, degradation, leakage, or stimulus-dependent release [30].

Transfer across lesions and settings is uncertain because primary tumors and metastases may differ in vascular, stromal, immune, and targeting properties [25]. Findings must therefore be validated across relevant cancers, models, treatment histories, doses, and manufacturing batches.

Not every program must measure every state or delivery gate. Measurement depth should match the therapeutic mechanism, uncertainty, and intended claim. CSATM is intended to identify the smallest defensible evidence set while preventing unsupported substitutions, such as treating bulk uptake as intracellular delivery. It should not guide clinical, regulatory, or manufacturing decisions without prospective validation.

CONCLUSION

The Cell-State-Aware Targeting Model reframes nanomedicine delivery around the recipient rather than the aggregate tumor. Its central proposition is that administered dose, tumor accumulation, cellular contact, internalization, payload release, active intracellular exposure, and pharmacological consequence are sequentially related but non-equivalent. By combining spatially situated cell states, interacting vascular, stromal, immune, and malignant compartments, dynamic target accessibility, resistant niches, treatment-induced transitions, and distributional cellular-dose metrics, the model provides a testable architecture for determining who actually receives the dose. Its proposed constructs are intended to improve mechanistic reasoning, experimental design, and evidence reporting, not to establish validated predictions or clinical rules. The framework remains conditional on formulation identity, tumor context, measurement validity, time, payload mechanism, and intended decision use. Its value must therefore be judged through prospective, orthogonal, and context-specific validation rather than through conceptual coherence or tumor-accumulation measurements alone.

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